Subsurface landfill leachate - home to complex and dynamic eukaryotic communities

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Summary

Research on natural attenuation in groundwater ecosystems has mainly addressed the presence and abilities of prokaryotic microorganisms to reduce the mass and toxicity of pollutants. The occurrence and possible roles of eukaryotes in contaminant biodegradation has so far largely been overlooked. The present thesis places an emphasis on the presence of eukaryotes in a polluted aquifer downgradient of a landfill (Banisveld, The Netherlands) and discusses their potential contribution to natural attenuation of contaminants. The structure of microeukaryotic communities in sediments and groundwater from this aquifer was examined in relation to bacteria community structure and environmental factors, particularly with the presence of landfill leachate pollution.

Chapter 1 provides an introduction to this thesis where a background on the importance of groundwater is presented, along with the threatening sources of groundwater pollution, of which landfills receive primary attention, and the possible ways of natural attenuation of contaminants in the subsurface. This chapter contains also a brief description of the research location and employed methodology.

The structure of sediment-associated eukaryotic communities was examined using culture-independent methods in **Chapter 2** in relation to bacteria community and the heterogeneity in environmental factors. Eukaryotes were detected all over the aquifer; their diversity was high and community structure complex. The eukaryotic community structure did not seem to relate to depth in the aquifer or distance from the landfill. The diversity in eukaryotic 18S rRNA genes decreased with sampling depth, and was significantly lower in polluted samples than in clean samples. The eukaryotic, and also bacteria, community profiles associated more to each other in polluted sediment samples than in clean samples. The bacteria community profiles obtained in surface

sediment samples clustered together and separately from those in subsurface sediments. The belowground bacteria profiles clustered location-specific. No significant autocorrelation of bacteria or eukaryotic communities in sediments was observed over 1-m depth intervals per sampling location. This may suggest that sampling should be performed at smaller scales than 1 m, if one aims at fully characterizing the microbial communities in heterogeneous aquifers like that from Banisveld. Spatial heterogeneity in sediment-associated microbial communities was apparent and seemed much larger than that in groundwater. Spatial heterogeneity can complicate the assessment of microbial community structure and functioning, but in turn it provides better opportunities for natural attenuation. While transported with the groundwater flow, the landfill leachate contaminants will pass over more types of microorganisms in a heterogeneous environment, and thus have greater chances of being degraded, in comparison with more homogeneous settings.

As the general fingerprinting method (18S rRNA gene based Denaturing Gradient Gel Electrophoresis) used in Chapter 2 cannot indicate which types of organisms are present, in **Chapter 3**, culture-independent phylogenetic analysis of 18S rRNA gene fragments and culturing were employed on six sediment samples belonging to the same set as used in Chapter 2. A diverse microeukaryotic community was revealed in five sediment samples obtained from 3 to 5 m depth along a transect (21-68 m) downgradient of the landfill, and from one reference location unaffected by the leachate. The microeukaryotic community consisted of heterotrophic nanoflagellates, yeast-like fungi and green algae. Fungal sequences, especially belonging to yeasts of the *Basidiomycota*, dominated most clone libraries. Sequences most closely related to *Ascomycota*, *Chytridiomycota* and *Zygomycota* had also been encountered. Green algae (*Chlorophyta*) were detected in polluted locations close (<30 m) to the landfill, and were believed to have been introduced in this aquifer by the percolating rain water or other surface recharges, as this type of green algae were not described to be capable of growth in absence of light. The bacterivorous nanoflagellate *Heteromita globosa* (*Cercozoa*) was retrieved in aerobic and anaerobic enrichments, and its sequences dominated the clone library derived from the polluted aquifer at 5 m depth, and 21 m downgradient of the

landfill. The presence and activity of these bacterivorous protists can be of high importance in bioremediation of polluted environments by predating on bacteria and subsequent recycling of limiting nutrients. The number of culturable eukaryotes ranged from 10^2 to 10^3 cells/g sediment, whereas culture-independent quantification revealed slightly higher numbers. Groundwater mesofauna was not detected in the aquifer; the absence of larger stygobiontic invertebrates was due to the fine aquifer sediments, lack of oxygen and presence of landfill leachate pollution. The food web in the polluted Banisveld aquifer is simple; the food chains are short consisting of prokaryotes and fungi as decomposers of organic matter and protozoa as primary consumers of prokaryotes, and top predators in this particular groundwater ecosystem.

While in Chapter 2 large spatial heterogeneity was apparent in sediment characteristics and related microbial community, in **Chapter 4** the spatial and temporal heterogeneities and dynamics of microbial communities were studied in groundwater samples, with the aim of identifying whether the variation of bacteria and eukaryotic communities in time and space related to the overall spatial and temporal hydrochemical changes. The core plume of pollution was hydrochemically rather stable in time; similar pollution levels were detected in the plume in the interval 1998-2004. The plume upper fringe appeared to have moved up to the surface, especially at distances greater than 48 m from the landfill. Complex and heterogeneous bacterial and eukaryotic communities were resolved using DGGE of 16S and 18S rRNA gene fragments. Over the period 1998 to 2004, large fluctuations were noted in the eukaryotic communities associated with polluted and clean groundwater. The bacterial profiles of polluted samples were more similar to each other than to those in clean groundwater in 1998 and 1999, but no longer in 2004. Unlike the eukaryotic profiles, the 1998 bacteria profiles in polluted groundwater samples were more related to each other than to those recovered from polluted samples obtained in 1999 and 2004. The temporal variation in bacteria and eukaryotic communities was greater than the spatial variation at all sampled locations in the aquifer. Pollution with landfill leachate seemed to have a smaller contribution to the distribution of microbial communities in the

aquifer, in comparison with temporal fluctuations and heterogeneity in their community structure and environmental settings.

The presence or protists (i.e. *Heteromita globosa*) was evident in contaminated part of the aquifer at Banisveld (Chapter 3), a role in bioremediation was suggested. **Chapter 5** of this thesis presents the combined influence of predation by another bacterivorous flagellate (i.e. *Ochromonas* sp. DS) and type of nutrient limitation on biodegradation of organic matter. Mathematical modeling, subsequently confirmed experimentally, indicated that predation by this flagellate can enhance carbon mineralization, and leads to higher overall activity (activity per unit sample), under nitrogen limiting conditions. Under carbon limitation, predation had a negative influence on organic matter degradation, in comparison with when predation was absent. The information obtained in this chapter can be of significance for the degradation of organic contaminants in oligotrophic ecosystems (e.g. aquifers), where nutrients like nitrogen or phosphorus become in general rapidly depleted. By their predation on bacteria, protozoa can recycle limiting nutrients and may stimulate indirectly pollutant biodegradation.

Chapter 6 of this thesis makes a synthesis and discusses the obtained results. The groundwater environment from Banisveld experienced pollution threatening by landfill leachate for over four decades. This ecosystem appears to be resilient to landfill leachate pollution; ecosystem resilience is described here as the ability of ecosystems to cope with pollution threatening, and recover to a functional state similar to that prior to disturbance. The polluted aquifer at Banisveld possesses the potential for biodegradation of pollutants. The large heterogeneity in sediment characteristics and microbial community structure can be favorable to natural attenuation. Degrading microorganisms (bacteria and fungi) are present and capable of degrading organic materials. Protozoan predation on bacteria and subsequent recycling of limiting nutrients, are factors that may enhance the biodegradation of organic pollution in the aquifer. Several ecological aspects may make however this groundwater ecosystem quite vulnerable to pollution. The groundwater ecosystem is usually oligotrophic, with communities little or not adapted to large fluctuations in environmental conditions in their

habitat. As the groundwater food webs are in general simple with short food chains, large organic charge like that in the Banisveld aquifer may lead to a disturbed ecosystem with unstable communities.